SUMMARY

TECHNOLOGIES FOR DETECTING HERITABLE MUTATIONS IN HUMAN BEINGS

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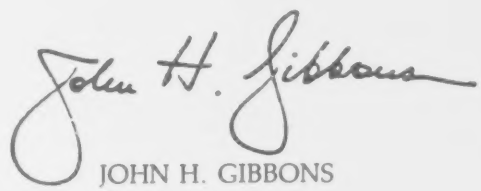
Foreword

Ensuring the health of future generations of children is of obvious importance to American society. Heritable mutations, permanent changes in the genetic material that can be passed on to succeeding generations, are the cause of a large but currently unquantifiable share of embryonic and fetal loss, disease, disability, and early death in the United States today. The methods now available to study heritable mutations, however, offer relatively little information about the kinds of mutations that can occur, their frequency, or their causes. Recent advances in molecular genetics have opened the door to new and innovative technologies that may offer a great deal more information about DNA. It may soon be possible to characterize mutations precisely, to measure their frequency, and perhaps also to associate particular mutations with exposures to specific mutagenic influences. While some of the new technologies are still on the drawing board, they are developing quickly and several of them may become available for wide-scale use in the next 5 to 10 years.

The Senate Committee on Veterans' Affairs, the House Committee on Science and Technology, and the House Committee on Energy and Commerce requested that OTA assess the available information about current and proposed means for detecting heritable mutations and on the likelihood and potential impact of such technological advances. These committees have wrestled with the problems of determining whether past exposures to potential mutagens have affected the health of Americans, and of framing reasonable public health laws, given current knowledge and technologies. This report summarizes OTA's findings as they relate to these issues.

An advisory panel, chaired by Arno G. Motulsky, provided guidance and assistance during the assessment. The OTA Health Program Advisory Committee, OTA staff, and scientific and policy experts from the private sector, academia, and the Federal Government provided information during the assessment and reviewed drafts of the report. We thank all who assisted us. As with all OTA reports, the content of the assessment is the sole responsibility of OTA and does not necessarily constitute the consensus or endorsement of the advisory panel or the Technology Assessment Board. Key OTA staff involved in the assessment were Michael Gough, Julie Ostrowsky, and Hellen Gelband.



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NOTE: OTA appreciates and is grateful for the valuable assistance and thoughtful critiques provided by the advisory panel members. The panel does not, however, necessarily approve, disapprove, or endorse this report. OTA assumes full responsibility for the report and the accuracy of its contents.

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[1]On detail from the National Institutes of Health, April-June 1985.

Technologies for Detecting Heritable Mutations in Human Beings

INTRODUCTION

Mutations, lasting changes in the genetic information carried in the deoxyribonucleic acid (DNA) of cells, can cause severe diseases and disabilities, none of which is curable and relatively few of which can be treated effectively. Such genetic diseases represent a significant fraction of chronic disease and mortality in infancy and childhood; they generally impose heavy burdens expressed in premature mortality, morbidity, infertility, and physical and mental handicap. Some of the most common of the 3,000 or more different disorders known to result from mutations include Down syndrome, Duchenne muscular dystrophy, and hemophilia. In addition, mutations have been associated with increased susceptibilities to certain chronic diseases, including some forms of diabetes, heart disease, and cancer. Most mutations that are expressed as genetic disease already exist in the population and are carried from generation to generation. A smaller proportion of mutations arises anew, "sporadically," in each generation, and the specific causes of these mutations are unknown.

The public and the government have expressed concern about the possibility that environmental exposures are contributing to or increasing the frequency of mutations. Mutations are among the chronic health effects singled out in the Toxic Substances Control Act of 1976 (TSCA) and in the Comprehensive Environmental Response, Compensation, and Liability Act of 1980 (CERCLA or "Superfund"). In those laws, Congress specified public protection from exposures that can cause mutations. The other major environmental statutes contain language broad enough to include protection from mutagens.

Unfortunately, little is currently known about the kinds and rates of mutations that occur in human beings. Available methods to study such mutations are inadequate to provide sufficient information for evaluating mutagenic risks.

Much of our knowledge of genetic risks to human health from exposures to environmental agents has been derived from the study of the effects of mutagens on experimental animals. These experiments are useful in manipulating various aspects of the mutagenic process, for example, to examine how mutagens act on DNA and to study effects of varying doses and rates of exposure to mutagenic agents administered either singly or in combination. Experimentation with animals is essential for assessing potential hazards of new chemical and physical agents before human populations have been exposed to them. However, at present, technical problems in detecting and measuring mutations limit animal experiments as they limit

human studies, so the results from animal experiments, using current methods, detect only a small proportion of the kinds and numbers of mutations that can occur.

Recent advances in molecular biology have led to the development of new technologies for examining DNA that may provide insight into the kinds and rates of mutations that occur in human beings. This report assesses these developments and discusses their potential for predicting risks of mutation from particular exposures.

At present, these new technologies propose reasonable and verifiable ways of detecting heritable mutations in human DNA and proteins, but none is efficient enough to be used on a large scale. However, there is considerable optimism in the scientific community that these new technologies can provide, for the first time, the means to obtain basic knowledge about the primary causes of mutation and the means to assess the kinds and rates of mutations that occur in human beings.

Data derived from studies in human beings, along with verifiable methods to extrapolate from corresponding animal data, will permit a more informed assessment of the medical and biological consequences of mutagenic exposures. At present, without such comparative data, it is difficult to know whether general extrapolations from animal data would lead to underestimates or overestimates of the genetic risks for humans. Continuing to rely on inadequate data may incur both human and financial costs, since conclusions drawn from this information contribute to decisions about acceptable levels of exposure and the level of society's resources that are devoted to providing protection from such exposures.

A combination of factors—concern that environmental exposures may be contributing to human mutations, questions about the fundamental nature of mutations, and increasing knowledge of the structure and function of DNA—increase the likelihood that new technologies will be developed and field tested. However, studies using these technologies may be expensive and will probably require the collaboration of a large number of scientists; their continued development, pilot testing, and large-scale application may require sufficient interest and financial support outside the scientific community. With such support, and with continued development of the techniques, some of these techniques could be ready for large-scale use in the next 5 to 10 years.

Congressional interest in supporting basic research on human mutations and in the continued development of these technologies is necessary if the regulatory agencies are eventually to have the tools to evaluate risks from most occupational or environmental exposures. The current lack of information on kinds and rates of human mutations is largely due to the inadequacy of present methods to study heritable mutations. Efforts to comply with the agencies' mandates to protect people from mutagens may be impeded unless basic knowledge of causes, kinds, and rates of human mutations is obtained.

Request for the Assessment

This assessment was requested by the Senate Committee on Veterans' Affairs, the House Committee on Science and Technology, and the House Committee on Energy and Commerce. Interest in the assessment was also expressed by the Senate Committee on Public Works and the Environment, the Senate Committee on Labor and Human Resources, and the House Committee on Veterans' Affairs.

These committees have wrestled with problems of determining whether past exposures to potential mutagens have affected the health of veterans and civilians and of framing reasonable public health laws that can be implemented, given current knowledge and technologies. OTA was asked to assess the available information about current means for detecting mutations as they relate to these issues and on the likelihood and potential impact of technological developments.

BACKGROUND

Kinds and Effects of Mutations in Human Beings

Mutations can occur "spontaneously," that is, in the apparent absence of any unusual stimuli, or they can be "induced" by particular agents. It is likely that many or most "spontaneous" mutations are caused by external forces, possibly including ionizing radiation, ultraviolet radiation, viruses, and certain chemicals, but the appropriate links have not been made. Some mutagens present around us may also be necessary for sustaining life, for example, oxygen, components of our food, and some of the body's own metabolites. Experiments in animals have shown that many substances present in agricultural, industrial, and pharmaceutical chemicals in use today are mutagenic in some test systems. Which of these cause mutations in human beings is still a matter of speculation. Precise causes for essentially all mutations that have been identified in human beings are unknown.

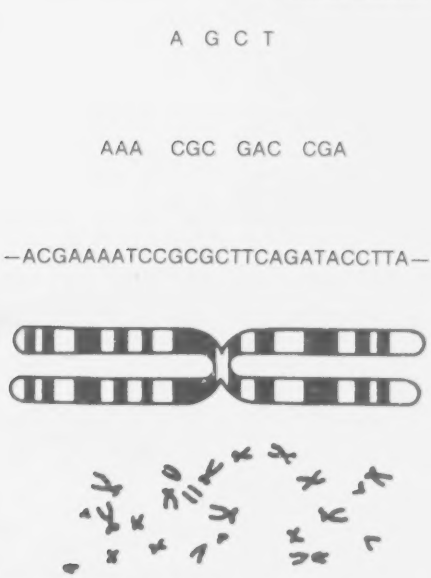
At present, more than 3,000 different genetic diseases have been identified, including disorders resulting from mutations in DNA, and disorders resulting from the interaction of genetic and environmental components. Approximately 10 in 1,000 liveborn infants are born with a single gene disorder and an additional 6 in 1,000 liveborn infants are born with a major chromosome abnormality. It is estimated that approximately 80 percent of the single gene disorders are the direct result of mutations that occurred in germ cells of distant ancestors and were passed along to succeeding generations. The remaining 20 percent of these cases (0.2 percent of all livebirths) and the majority of chromosome abnormalities are believed to be due to sporadic mutations in the reproductive cells of one of the parents of the infant. An additional 10 in 1,000 liveborn infants manifest a serious genetic disease sometime after birth, and a far higher proportion of newborns will show indirect effects of one or several parental or ancestral mutations in later life as, for example, in increased susceptibilities to some forms of heart disease, diabetes, or cancer.

Mutations are changes in the composition of the genetic material, DNA (see fig. 1), and are generally divided according to size into gene mutations and chromosome mutations. Gene mutations refer to changes within a single gene, for example, substitutions of single component nucleotides, or small losses or additions of genetic material in expressed or nonexpressed regions of the gene. Chromosome mutations affect larger portions of the chromosome (e.g., structural rearrangements of genetic material in the chromosomes) or result in the loss or addition of an entire chromosome. Since DNA directs the synthesis and regulation of molecules in the body, either group of mutations can influence a wide range of biological and physiological functions, including reproduction, longevity, intelligence, and physical development. Individual differences in susceptibility to disease may result from the effects of one gene, several genes, or combinations of genes and environmental factors.

Depending on the nature and location of the mutations and on the function of the genes in which they occur, mutations may, in theory, be beneficial, neutral, or harmful to the individual.[1] The kinds and effects of known mutational events range from single nucleotide substitutions (the smallest unit of change in DNA) with no clinically observable effects, to single nucleotide substitutions resulting in severe diseases; from major structural and numerical chromosome abnormalities (the largest observed unit of change in DNA) leading to various abnormalities and impairments, to those resulting in embryonic, fetal, or neonatal death.

[1]Current theory maintains that *most* newly arising mutations in regions of DNA that directly determine the structure and regulation of proteins are more likely to be detrimental than beneficial. Little is known of effects of mutations in other regions of DNA.

Figure 1.—Organizational Hierarchy of DNA, the Carrier of Genetic Information in Human Cells



Nucleotides
Adenine, guanine, cytosine, and thymine, the basic building blocks of DNA.

Codons
Nucleotides arranged in a triplet code, each corresponding to an amino acid (components of proteins) or to a regulatory signal.

Genes
Functional units of DNA needed to synthesize proteins or regulate cell function.

Chromosome
Thousands of genes arranged in a linear sequence, consisting of a complex of DNA and proteins.

Genome
The complete set of genetic information; each human reproductive cell contains 23 chromosomes, and all other cells in the body contain a full set of 46 chromosomes.

SOURCE: Office of Technology Assessment.

A child's entire genetic endowment comes from the DNA of two single reproductive cells (or gametes), one egg and one sperm, from his parents.[2] A mutation occurring in the DNA of either of these germ cells, a *germinal mutation*, is passed on to the child, who is born with a "new" *heritable mutation*. Mutations in germ cells that are not involved in fertilization are not passed on to the offspring. If a mutation arises in the DNA of the parents' nonreproductive cells (collectively termed somatic cells), such *somatic mutations* are not transferred to the reproductive cells. Somatic mutations may, however, affect the parents' health, and indirectly, their ability to bear a healthy child.

CURRENT METHODS FOR STUDYING MUTATIONS

Current empirical methods to study mutations in human beings focus on physiological and biochemical effects of mutations because, until recently, it was not possible to examine changes in DNA directly. Each of the current methods detects only a limited portion of the spectrum of mutational changes. These methods have been used to derive estimates of baseline frequencies of some kinds of human mutations.

Animal Studies

Much of our knowledge about how substances interact with DNA and how they may cause mutations is derived from studies with experimental animals. In addition, some estimates of human mutation rates have been derived, by extrapolation, from animal studies. Beginning soon after World War II and still continuing, spontaneous and induced gene mutation rates have been studied in laboratory mice. The rate of spontaneous heritable gene mutations, as detected in these experiments, is roughly two to eight mutations per 1 million genes per generation of mice. Radiation and approximately two-thirds of about 20 chemicals[3] tested so far increase the frequency of detectable heritable gene mutations in these experimental mice. Generally, the chemical substances that induce heritable mutations in mature and maturing germ cells also induce somatic mutations, and vice versa. However, substances that induce somatic mutations do not necessarily induce heritable mutations in immature germ cells (stem cells). Unlike mature germ cells or somatic cells, these germinal stem cells may have efficient systems for repair of mutational or premutational damage. There is, in fact, indirect evidence from dose-response and dose-rate experiments that germinal stem cells have good repair systems.

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[2]Each of the gametes (the male spermatozoan and the female ovum) is the product of a series of developmental stages of reproductive (or germ) cells.

[3]On the basis of animal experiments using the specific locus test and the heritable translocation test, in which they were found to be mutagenic in all germ cell stages, these chemicals are strongly suspected to be mutagenic in humans (U.S. Environmental Protection Agency, "Proposed Guidelines for Mutagenicity Risk Assessment," *Federal Register* 9(227):46314-46321, Nov. 23, 1984). They included both common environmental agents and chemicals available in the laboratory that are not normally found in the environment.

It may be useful to quantify relationships between somatic and germinal cells with regard to mutagenic potency of different chemicals, and to study mutations in equivalent sets of genes in both types of cells. Animal experimentation is useful for determining the feasibility of using human somatic mutation rates for predicting the risk of human heritable mutations. This work in animals may demonstrate whether it is possible to extrapolate from the occurrence of somatic to that of heritable mutations at all, and may help determine whether it is possible to generalize from animal data to human beings. In addition, animal studies on heritable mutations are useful not only for determining whether a given agent is mutagenic but also for more general explorations of the factors that may influence the occurrence of mutations.

Studies in Human Beings

Spontaneous heritable mutations in human beings have been studied by examining: 1) the incidence of certain genetic diseases ("sentinel phenotypes"), 2) gross changes in chromosome structure or number, and 3) changes in the structure or function of blood proteins. Epidemiologic studies of specific populations, in particular, the survivors of atomic bombs in Japan, provide some information about induced heritable mutations in human beings.

Sentinel Phenotypes

The classic method for identifying human heritable mutations is the empirical observation of infants and children for the presence of certain rare genetic diseases known as sentinel phenotypes. Examples include achondroplasia (dwarfism), aniridia, and some childhood cancers, such as retinoblastoma and Wilms' tumor. By recording the occurrence of sentinel phenotypes as a proportion of the total number of livebirths in a defined population over time, the frequency of each disorder (and of its corresponding mutation) can be estimated.

The characteristic of sentinel phenotypes that is most useful for mutation studies is that these conditions are "sporadic" in most or all cases; they almost always result from a new germinal mutation in one of the parents of the afflicted individual.[4] Each different sentinel phenotype is thought to result from a different, single, mutant gene, although precise genetic information to confirm the single gene hypothesis is lacking in most cases.

Despite the distinctive characteristics of sentinel phenotypes, the relevance of existing data on the frequency of the various sentinel phenotypes to the study of kinds and rates of human mutations is limited by a lack of knowledge of the genetic bases of the phenotypes, and by the small fraction of DNA that accounts for these phenotypes. Of the several thousand known genetic diseases, only 40 are thought to satisfy the criteria for inclusion as "sentinel phenotypes." Roughly 40 genes are involved in the 40

[4]Various tests are done to exclude nonsporadic cases, which could result from X-linked recessive inheritance, mistaken parentage, and the occurrence of other genetic or nongenetic conditions that mimic the appearance of sentinel phenotypes.

sentinel phenotypes, among a total of an estimated 50,000 to 100,000 expressed genes in an individual's DNA.

Sentinel phenotypes are severely debilitating conditions that require accurate diagnosis and long-term medical care. However, practical difficulties arise in gathering and maintaining data on the incidence of sentinel phenotypes for the purpose of tracking mutation rates. Infants with sentinel phenotypes are rare, numbering approximately 1 in 10,000 to 1 in 10 million liveborn infants, depending on the particular disease. Consequently, a huge number of infants must be observed in order to find even a few infants with sentinel phenotypes. Diagnosis of individual phenotypes is complicated by the genetic heterogeneity of these disorders, so that highly trained specialists in various pediatric subdisciplines, which are few in number, would be needed to make these observations. Millions of consecutive newborn infants would have to be monitored thoroughly and accurately for many years, and registries, much larger than those currently in use, would be needed to collect and maintain the necessary data.

Chromosome Abnormalities

Another method for identifying a certain class of human heritable mutations is the examination of chromosomes under a light microscope for the presence of chromosome abnormalities ("cytogenetic analysis"). Normal human DNA is organized into 46 chromosomes (44 autosomes and 2 sex chromosomes), distinguishable by size, proportional shape, and staining pattern, or "banding." Chromosome abnormalities are defined as either *numerical* (extra or missing whole chromosome[s]) or *structural* (deletions, insertions, translocations, inversions, etc., of sections of chromosomes). In total, chromosome abnormalities are estimated to occur in at least 5 percent of all human conceptions. The majority of such conceptuses are spontaneously aborted, but the few that survive comprise approximately 0.6 percent of all liveborn infants. The incidence of Down syndrome is 1 in 650 at birth, making it the most common chromosomal disorder in newborns.

With the most advanced chromosome staining methods currently available, approximately 1,000 bands are distinguishable in one set of human chromosomes, although far fewer bands are produced with routine methods. Mutations in DNA sometimes cause a change in the banding pattern, particularly if such mutations involve large sections of a chromosome. With routine banding methods, there may be several hundred genes present in each visible band, and with higher resolution banding methods, a single band may contain about 100 genes. However, smaller mutations, from single nucleotide changes within genes on up to some deletions and insertions of entire genes, generally are not visible by any banding method.

Measurement of Mutant Proteins

Most of the available information on rates of spontaneous human mutations has been derived from studies of sentinel phenotypes and chromosome abnormalities, but data on different kinds of mutations in humans

is now emerging from studies of mutant proteins. In general, mutant proteins are more precise indicators of genetic damage than are clinical and cytogenetic observations. Certain mutations in genes that determine the structure of proteins alter the chemical characteristics of the proteins, causing them to behave differently in separation and purification procedures. These differences suggest that a mutation has occurred because proteins are constructed according to blueprints in DNA, and changes in DNA can lead to the production of altered proteins. If the protein under study is an enzyme, a mutation within the gene that codes for it can alter, diminish, or eliminate the enzyme's biochemical activity.

Operationally, mutant proteins are identified by taking samples of blood from each member of a "triad," including both parents and the child. Proteins are extracted from blood components, and the proteins are separated by electrophoresis. Putative mutations are identified when a protein from a child behaves differently from the corresponding protein from both parents.

One-Dimensional Separation of Proteins.—The technique most commonly used to study mutant proteins is electrophoresis, a method of separating proteins on the basis of their electrical charges. The term "electrophoretic variant," or "electromorph," is used to describe a protein that behaves differently in electrophoresis from the corresponding protein found in the parents.

Although one-dimensional electrophoretic analysis of proteins is well established and can be improved by including functional assays for additional enzymes, it is limited to detecting: 1) mutations that do not eliminate the functional ability of the proteins, 2) nucleotide substitutions only in coding regions of genes for the proteins examined, and 3) only those nucleotide substitutions that alter the electrical charge on these proteins; such substitutions are thought to account for about one-third of all nucleotide substitutions in coding regions, which, in turn, account for a fraction of all the kinds of mutations that can occur. Electrophoresis does not detect many other types of mutations, including small duplications, rearrangements, or mutations that result in the absence of gene products; these mutations are thought to constitute the majority of the mutations induced by certain mutagens, including radiation. Moreover, electrophoresis does not detect mutations that occur anywhere outside the coding regions of a certain set of genes, including mutations in other coding genes and in nonexpressed regions of the DNA.

Data from several studies using one-dimensional electrophoresis have been used to estimate the rate at which mutations produce electrophoretic variants, and from this estimate, to infer the total rate of amino acid substitutions in proteins, and the corresponding mutation rate per nucleotide in human DNA.

Two-Dimensional Separation of Proteins.—An extension of one-dimensional electrophoresis involves separation of proteins in a second dimension. With two-dimensional electrophoresis, about 300 proteins from

each person can be separated and examined, compared with about 100 proteins per sample that can be separated in one-dimensional electrophoresis. Further improvements may be possible with the use of computer algorithms to assist in interpreting the complex two-dimensional gels. This technique is currently feasible, and although it detects the same types of mutations as one-dimensional electrophoresis, it can examine more proteins per sample.

Epidemiologic Studies

An extensive body of data from experimental animals demonstrates that exposure to radiation and to certain chemicals can induce mutations in mammalian germ cells. In humans, exposure to ionizing radiation is known to cause somatic mutations, and it is suspected to enhance the probability of heritable mutations. *To date, however, the available methods have provided no direct evidence for the induction (by chemicals or by radiation) of mutations in human germ cells.*

The single largest population studied for induced mutations is the group of survivors of the atomic bombs detonated in Hiroshima and Nagasaki in 1945. Many survivors of the bombs received doses of radiation that could have caused germinal mutations; in experiments with mutation induction by radiation in mammals, similar kinds and doses of radiation were sufficient to cause observable mutations in offspring. Therefore, it was assumed that germinal mutations could have been induced in people exposed to the radiation from the blasts.

Medical examinations of the survivors soon after the blasts revealed the immediate effects of whole body irradiation: loss of hair; reduction in bone marrow activity; and reduction in circulating white blood cells, associated with a reduction in the body's resistance to infection. Among those who recovered from the immediate effects of the radiation, there was a significant excess of cancer deaths later in life. Certain types of leukemia were the first cancers to appear in excess, but continuing followup has revealed later increases in other cancers, such as multiple myeloma and cancers of the breast, thyroid, colon, esophagus, stomach, lung, ovaries, and possibly of the spinal cord and nerves.[5,6]

Exposure of pregnant women to radiation was found to be associated with an increased incidence in their liveborn infants of small head circumference, mental retardation, and an increased incidence of childhood cancers. The critical time for fetal brain damage from radiation exposure was identified as the period of 8 to 15 weeks of gestation.[7]

Analysis of the chromosomes prepared from peripheral blood lymphocytes of survivors exposed to the radiation has indicated an excess of chro-

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[5]S. Darby, E. Nakashima, and H. Kato, "A Parallel Analysis of Cancer Mortality Among Atomic Bomb Survivors and Patients With Ankylosing Spondylitis Given X-Ray Therapy," *J. Nat. Cancer Inst.* 75(1):1-19, 1985.

[6]H. Kato, and W.J. Schull, "Cancer Mortality Among Atomic Bomb Survivors," *Life Span Study Report 9, Part 1*, Radiation Effects Research Foundation Technical Report 12-80, 1982.

[7]M. Otake, and W.J. Schull, "In Utero Exposure to A-Bomb Radiation and Mental Retardation: A Reassessment," *Brit. J. Radiol.* 57:409-414, 1984.

mosome aberrations.[8] Certain types of chromosome aberrations (mainly balanced structural rearrangements, such as reciprocal translocations and inversions) have been found to persist in circulating lymphocytes long after exposure to radiation, whereas other types of chromosome aberrations (e.g., unbalanced rearrangements) in lymphocytes declined in number soon after exposure. Overall, the frequency of chromosomally aberrant cells in the survivors' blood was found to be proportional to the estimated dose of radiation received at the time of the bombing. It has not yet known whether these somatic mutations are correlated with specific cancers or other diseases in the survivors.

Survivors' children who were conceived after the acute radiation exposure were examined to study mutagenic effects on the parents' reproductive cells. Using various methods available from 1945 to the present, survivors' offspring were studied for "untoward pregnancy outcomes,"[9] for certain chromosome abnormalities, or most recently, for abnormal blood proteins. The offspring of parents exposed to atomic radiation were compared with the offspring of parents who were beyond the zone of radiation (greater than 2,500 meters from the hypocenter at the time of the bombings). Observation and analysis of some 70,000 offspring has revealed no statistically significant excess in the incidence of stillbirths, congenital malformations, neonatal deaths, or chromosome abnormalities. These findings suggest that the frequency of radiation-induced germinal mutations that led to certain gross abnormalities in newborn infants was not high enough to be detectable in a population of that size and genetic heterogeneity. However, they do not rule out the possibility of other manifestations of genetic damage in these children, or of latent expressions of such damage, since the methods used to study this population could examine only a small subset of DNA and only a limited number of genetic endpoints.

Analysis of the children's blood proteins for electrophoretic variants was later done to detect recessive mutations, that is, mutations that are not expressed as disease (unless present in both copies of a particular gene). This analysis, begun in 1976, found few mutations in either the exposed or control groups, making interpretation problematic. While the results indicate no significant excess of mutant proteins in the children of exposed parents, they do not exclude the possibility that an excess exists undetected. Unfortunately, electrophoresis is inefficient at detecting deletions, one of the most likely types of radiation-induced mutations. Overall, these findings do not rule out the possibility of genetic damage to the offspring of survivors of the atomic bombs, but they put upper limits of the frequency of occurrence of certain types of mutations that the current methods are able to detect.

Taking these findings at face value, and cognizant of an enormous body of data on the genetic effects of radiation on experimental animals, the in-

[8]A.A. Awa, T. Sofuni, T. Honda, et al., "Preliminary Reanalysis of Radiation-Induced Chromosome Aberrations in Relation to Past and Newly Revised Dose Estimates for Hiroshima and Nagasaki A-Bomb Survivors," Radiation Effects Research Foundation Technical Report Series 8-83, 1983.

[9]These were defined as major congenital defects and/or stillbirths and/or death in the survivors' offspring during the first postnatal week. These abnormalities can be caused by exposure to radiation, as well as to other environmental agents, and by socioeconomic factors.

vestigators suggest that the dose of radiation necessary to double the human mutation rate (the "doubling dose") was between 139 and 258 rem,[10] but they caution that there is a possibility of large error attached to that estimate since genes other than the ones sampled may demonstrate different sensitivity to radiation, and some types of mutations may be repaired more efficiently than others. Their estimate of the doubling dose, if correct, however, indicates that man could be considerably *less* sensitive to radiation than laboratory mice.

NEW TECHNOLOGIES FOR DETECTING HUMAN MUTATIONS

Recent advances in molecular biology have led to the development of techniques that allow direct examination of DNA for evidence of mutations. These methods can examine large regions of human DNA without requiring detailed knowledge of the genes contained in those regions and without preparing genetic probes for particular sequences. Unlike current methods—observing sentinel phenotypes, chromosome abnormalities, and electrophoretic variants—that are limited to detecting a small fraction of all kinds and numbers of mutations, the new techniques have the potential for detecting a wide, unselected spectrum of mutations across the DNA. These techniques are examples of the state-of-the-art in molecular genetics and they are now promising to provide the basis for better approaches to studying mutagenesis.

Applying recent developments in molecular biology to the problem of detecting sporadic mutations, the new techniques described in this report propose reasonable and verifiable ways of examining human DNA for alterations in sequence and structure. These developments include the ability to clone specific genes, to cut up DNA into predictable fragments, to hybridize complementary DNA strands, to detect less-than-perfect hybridizations due to single base pair changes, and to separate large fragments of human DNA. Some of these methods detect similar types of mutations and some complement each other by detecting different types. Some of these may merit further development and, eventually, pilot testing. Several new technologies, representing different approaches, are discussed below.

None of these new techniques has been applied to large human populations or to experimental animals and it is not known how well they will perform. At present, none of these techniques is approaching the efficiency needed for examining the kinds and rates of mutations in a population or for determining whether mutation rates are increasing. With technical improvements in efficiency, some of the techniques, or derivatives of them, could be available in the next 5 to 10 years for large-scale use. Since these technologies provide new information about DNA, the health implications of any newfound mutations may not be immediately known. Additional

[10] A rem (Roentgen-Equivalent-Man) is a measure of absorbed radiation dose. For comparison, a chest X-ray exposes an individual to about 0.1 rem.

research and methods would be needed to examine biological and physiological implications of the identified mutations for the populations studied and for their descendants.

Detection and Measurement of Heritable Mutations

Restriction Fragment Length Polymorphisms

Restriction endonucleases are enzymes, isolated from bacteria, that can be used experimentally to cleave isolated DNA molecules into fragments at specific sites in the DNA sequence that they recognize. If any of these sites have been altered by mutation, the resulting pattern of fragment sizes would also be altered. Restriction enzymes can be used to detect mutations that either: 1) create a new restriction site, 2) eliminate an old one, or 3) change the distance between existing restriction sites. (These mutations may include single nucleotide substitutions, or multiple nucleotide deletions or insertions.)

To use this method to detect mutations, DNA would be treated with a set of restriction endonucleases and the resulting DNA fragments separated by electrophoresis and examined for differences between those present in the child's DNA and those in either parent's DNA. Restriction site analysis does not allow examination of every nucleotide. However, the use of a set of combined restriction enzymes increases the number of restriction sites identified, allowing a larger portion of the DNA to be examined, including both expressed and nonexpressed regions.

Genomic Sequencing

The most straightforward approach to looking for mutations is by determining the sequence of every nucleotide in a child's genome and then comparing this with the DNA sequences of the child's parents. To determine its sequence, human DNA is cut with restriction endonucleases into fragments, and then each fragment is analyzed for its sequence of nucleotides. Genomic sequencing would detect mutations regardless of where they occur—in regions that code for specific proteins and regulatory functions as well as in regions without known functions—and is therefore potentially very informative. While it is technically possible at present, sequencing is currently feasible only for very limited sections of DNA, such as the length of DNA comprising only a few genes. Because of current technical inefficiencies, it would be an enormous task, involving many laboratories, a large number of scientists, and at least several decades to sequence even one entire genome, the complete set of DNA in an individual germ cell. At present, it is not feasible to use genomic sequencing to examine several peoples' genomes for mutations, although sequencing can be used in conjunction with other techniques to examine small sections of DNA.

One-Dimensional Denaturing Gradient Gel Electrophoresis

A modification of the standard electrophoretic gel procedure, denaturing gel electrophoresis allows DNA fragments to be separated not only on the basis of size, but also on the basis of sequence of nucleotides. Double-

stranded DNA separates ("denatures") into its constituent strands when it is heated or when it is exposed to denaturing chemicals. A gradient of increasing strength of such chemicals can be produced in an electrophoretic gel so that DNA samples will travel in the direction of the electric current, separate by size, and begin to dissociate as they reach their particular critical concentration of denaturing chemical.

Every unique strand of DNA dissociates at a different concentration of denaturant. In fact, a sequence difference of only one nucleotide between two otherwise identical strands of double-stranded DNA is enough to cause the strands to dissociate at different concentrations of denaturant chemical, and to stop traveling at different locations in the gel. Using this technique, the parents' and child's DNA would be cut into fragments with restriction enzymes, dissociated into single stranded DNA, and reannealed with radioactively labeled probe DNA. The resulting heteroduplex fragments would then be separated on the basis of their DNA composition in a denaturing gradient gel. Mismatches between the sequences of probe and child's DNA and not between probe and parents' DNA would appear as different banding patterns on the gel. Again, a comparison between the banding pattern of parents' and child's DNA analyzed in this way may identify a wide range of mutations in all DNA regions.

Two-Dimensional Denaturing Gradient Gel Electrophoresis

Another approach to detecting mutations is a technique whereby sizing and denaturing gels are used to differentiate among DNA sequences common to parents' and child's DNA, polymorphisms in either parent's DNA which are transmitted to the child, and any new mutations in the child's DNA. Like the two-dimensional polyacrylamide gel procedure for protein separation described earlier, this approach compares locations of spots on a gel (in this case, DNA spots) for evidence of new mutations. In this method, various combinations of parents' and child's DNA are produced and compared on the basis of the denaturant concentration at which they dissociate. Parents' and child's DNAs are compared to each other, rather than to relatively small probes. This approach, which would allow detection of mutations in the complete genome, would detect differences ("mismatches") between a child's DNA and his or her parents' DNA. Such mismatches would represent various types of mutations in the nucleotide sequence in expressed and nonexpressed DNA regions.

DNA-RNA Heteroduplex Analysis

This technique hinges on the production of DNA bound to complementary strands of ribonucleic acid (RNA) or "DNA-RNA heteroduplexes," and on the use of specific enzymes, such as "RNaseA," that cleave the DNA at particular sequences where the DNA and RNA are not perfectly bound at every nucleotide. This is similar to the use of restriction enzymes, which cleave DNA at particular normal sequences, except that RNaseA cleaves the RNA strand in RNA/DNA hybrid molecules where there are mismatched nucleotides, indicating mutations. The resulting fragments are then sepa-

rated electrophoretically to detect differences between parents' and child's DNA. The efficiency of this approach depends on the number of different mismatches that can be recognized and cleaved. This method would detect nucleotide substitutions over a large portion of the DNA.

Subtractive Hybridization

Detecting mutations would be much easier if it were possible to ignore the majority of DNA sequences that are the same in parents and child and, instead, focus only on the few sequences that may be different. Subtractive hybridization is an idea for selecting and characterizing sequences in a child's DNA that are different from either parent's DNA.

First, the double-stranded DNA of both parents is cut into fragments with restriction enzymes, dissociated into single-stranded DNA, and then mixed together with a set of single-stranded reference DNA sequences. These reference sequences represent all possible sequences of 18 nucleotides (analogous to a dictionary of 18-letter words using only 4 different letters.) Each reference sequence binds to its complementary sequence in the parental DNA and can be removed from the mixture. Any reference sequences left over, not bound to parental DNA, represent sequences not found in the parents' DNA. If the child's DNA binds with any of these left-over sequences, such hybrids would indicate that the child's DNA contains different sequences from those in the parents' DNA. These hybrids could be separated and analyzed for mutations.

This approach is the least well developed of all the ones discussed in this report, and its feasibility is unknown. If it does prove feasible, however, this approach would identify short sequences containing mutations in any part of the DNA, allowing further detailed study (e.g., by DNA sequencing) of the kinds of mutations that may occur.

Pulsed Field Gel Electrophoresis

If human DNA were short and simple, it could be cut up with restriction enzymes and separated electrophoretically into discrete bands, each representing a particular segment of the total DNA. However, human DNA is so long that when restriction-digested DNA is electrophoretically separated, the resulting fragments of the whole set of DNA form a continuous smear of bands. Even if the DNA could be cut into 100 or 200 fragments, the pieces would be too big to pass individually through the pores of a standard electrophoretic gel. A new technique, pulsed field gel electrophoresis is being developed to separate large fragments of human DNA and to examine such fragments for evidence of mutations. The procedure may detect submicroscopic chromosome mutations, including rearrangements, deletions, breaks, and transpositions. At the present time, however, the method cannot handle whole human chromosomes, though it works well with fragments of human chromosomes and with smaller whole chromosomes from lower organisms. This technique may be useful in detecting chromosome mutations that are intermediate in size between major rearrangements (ob-

servable by cytogenetic methods) and single base pair changes, potentially a large proportion of all possible mutations.

Detection and Measurement of Somatic Cell Mutations

The methods for detecting heritable mutations rely on comparing the DNA of parents with the DNA of their children to infer the kinds and rates of mutations that previously occurred in parents' reproductive cells. While this information is valuable in learning about heritable mutations, it may come months or years after the mutations have actually occurred, and this temporal separation of events makes it difficult to draw associations between mutations and their causes. Tests to detect *somatic* mutations may be useful in signaling the probability of heritable mutations. Such tests may be useful in relating exposures to specific mutagens with particular genetic events in the cell, and they may help to identify individuals and populations at high risk for mutations.[11]

It is thought that the mutation process is fundamentally similar in germinal and somatic cells. If this is true, then it may be possible to predict the risk of germinal and heritable mutations on the basis of measurements of somatic mutations, which are inferred from the frequency of mutant cells. Several investigators are currently working on methods to relate the frequency of mutant cells to the number and kinds of underlying mutations.

Several new techniques for detecting and measuring somatic mutations are described in this report. Mutant somatic cells may occur during growth and development and may appear as rarely as one in a million normal cells. Detection of somatic mutants requires methods for scanning through a million or so nonmutant cells to find a single variant cell. Two general approaches are used: 1) screening, which uses high-speed machinery to look at the total population of cells and either count or sort out the variants; and 2) selection, in which a population of cells is cultured in the laboratory under conditions that permit the growth of variant cells and that restrict the growth of the majority of cells that are nonvariants. The new DNA technologies could be used to characterize the mutations in any such somatic variants found.

Different mutagens have been shown to produce distinguishable types of mutations ("mutational spectra") in human cells grown in culture. Determining mutational spectra may be useful in associating specific mutational changes in somatic cells with particular mutagenic agents to which individuals may be exposed. Such information could be useful in understanding the causes of mutational changes as well as in monitoring at-risk populations.

[11]Even without knowing the exact relationship between rates and kinds of somatic and heritable mutations, it is reasonable to predict that people with high somatic mutation rates might be at higher risk for heritable mutations, either because of a particular environmental exposure or a genetic susceptibility to mutations.

Data on kinds and rates of somatic mutations may provide a monitor for exposure to mutagens and carcinogens, and are relevant to the study of carcinogenesis and of aging. However, measurements of somatic mutation rates per se have little direct applicability to intergenerational (or transmitted) effects, without corresponding information on heritable mutations.

USE OF NEW TECHNOLOGIES IN RESEARCH AND PUBLIC POLICY

Feasibility and Validity Testing

The new technologies now range from ideas on paper to being in various stages of laboratory development, but none is yet ready for use in the field. A critical step before a technology is used in an investigation of a population thought to be at risk for mutations is that the technology be tested for validity and feasibility. To assure that a technology is a "valid" method, that is, that it detects the types of mutations that it theoretically is capable of detecting, and to characterize the degree to which it gives the "right" answer, it will be necessary to test a technology in a series of validation studies. A first step might be to test the technologies against pieces of DNA with known mutations, and at a later stage, in offspring of animals exposed to known mutagens. Feasibility testing will be required to make sure the technology can be efficiently scaled up for analyzing large numbers of samples.

Epidemiologic Activities

If the value of new technologies for detecting mutations is to be realized, it will be as tools for determining rates and patterns of mutations in epidemiologic studies of human beings. Once a technology has successfully passed through validation and feasibility tests, it will become a candidate for use in three major types of epidemiologic activities: surveillance, monitoring, and ad hoc studies.

Surveillance is a routine activity whose aim, in the context of this report, would be to measure the "baseline" rate of mutations in a defined population over the course of time and to facilitate rapid recognition of changes in those rates. *Monitoring* consists of observations over time in a population thought to be at increased risk of, in this case, heritable mutations, because of exposure to a known or suspected mutagen, for the purpose of helping the specific at-risk population in whatever way is possible. People living around hazardous waste disposal sites have been monitored for endpoints other than mutations, e.g., cancer and birth defects, and they would be likely candidates for mutation monitoring when technologies become available to do so efficiently. *Ad hoc studies* of a variety of designs are carried out to test hypotheses about suspected causes of mutations. Ideally, the results of ad hoc studies can be generalized to populations other than those specifically studied.

Extrapolation

Making predictions from observations of cause and effect in one system to probable effects in another is one form of extrapolation. The process involves a set of assumptions in moving from one system to another. The practical importance of extrapolation for mutagenicity is, ideally, to be able to predict mutagenic effects on human beings from the response in laboratory animals or lower test systems. The ability to extrapolate to human responses addresses one of the major goals of public health protection, the ability to identify substances harmful to human beings before anyone is exposed, thereby providing a rational basis for controlling exposure.

Extrapolation can be qualitative or quantitative. Qualitative, also called biologic, extrapolation involves predicting the *direction* of a result, for example, if a chemical causes mutations in a laboratory test, can we also expect mutations in human beings? Quantitative extrapolation involves translating a quantitative result in an animal test into a quantitative estimate of mutagenic risk in humans. Going a step further in extrapolation, can an estimate of mutagenic damage be translated into a measure of genetic disease?

A number of theoretical models for extrapolating mutagenic effects have been proposed, based on various parallel relationships. For instance, it might be true that if the relationship between somatic and heritable effects in animals were known after exposure to a specific mutagen, and if one could measure a somatic effect in human beings who had been exposed to the same substance, a heritable effect in human beings could be predicted, assuming the relationship between somatic and heritable effects is parallel in animals and human beings. Because of the paucity of data, particularly from human studies, it has been impossible to validate such an extrapolation model. The new technologies should allow a major increase in the database which, in turn, should allow researchers to more fully explore relationships among various types of test results.

Regulation

Congress has mandated public protection from mutagens in certain environmental health laws (e.g., TSCA and CERCLA), and other laws provide mandates broad enough to empower agencies to take action against mutagens in virtually any appropriate situation. Except for radiation, however, very little regulatory evaluation has taken place on the subject of heritable mutations. This is directly related to the lack of sensitive methods to detect heritable mutations in human beings, and the related difficulty in extrapolating from results in nonhuman test systems to probable human responses.

The Environmental Protection Agency (EPA) has recently issued "Guidelines for Mutagenicity Risk Assessment." EPA's approach is relatively simple and pragmatic. It requires only the types of information that can be acquired with current technologies, but allows for information from new technologies, as they become available. The guidelines require evidence of:

1) mutagenic activity from any of a variety of test systems, and 2) chemical interactions of the mutagen in the mammalian gonad. Using a "weight-of-evidence" determination, the evidence is classified as "sufficient," "suggestive," or "limited" for predicting mutagenic effects in human beings.

Although chemicals have not generally been regulated as mutagens, it is probable that exposures to mutagens have been reduced by regulations for carcinogenicity. Strong evidence supports the idea that a first stage in many cancers involves mutation in a somatic cell, and one of the most widely used screening tests for potential carcinogens, the Ames test, is actually a test of mutagenicity. The extent to which people are protected against heritable mutations if their cancer risk from a specific agent is minimized is at present unknown. The new technologies should greatly improve our ability to make that judgment.

Federal Spending for Mutation Research

OTA queried Federal research and regulatory agencies about their support of research directed at understanding human mutations. For fiscal year 1985, they reported about $14.3 million spent on development or applications of methods for detecting and/or counting human somatic or heritable mutations. An estimated $207 million was spent in the broader category of related genetic research.

OPTIONS

Research related to the new technologies described in this report has the dual aims of increasing the knowledge base in human genetics about the causes and effects of mutations, and producing information that could be used to estimate mutagenic risks for the purpose of protecting public health. The pace and direction of research toward developing these methods and the quality and efficiency of preliminary testing of methods could be influenced by congressional and executive branch actions and priorities. Integration of research in different test systems and progress in developing extrapolation models also can be influenced by actions now and in the near future.

Continued progress in the development and application of new technologies will depend not only on support of the individuals and laboratories directly involved in this research, but also on work in other areas. Although not directly addressed in this assessment, support of research in medical, human, mammalian, and molecular genetics will be essential to a full understanding of the mutation process.

The options that follow are grouped in four sections: 1) options to influence the development of new techologies, 2) options to address various aspects of feasibility and validity testing, 3) options concerning the use of new technologies in field studies, and 4) options to encourage coordination of research and validation of extrapolation models.

Development of New Technologies

The Department of Energy (DOE), several agencies of the Department of Health and Human Services, and EPA currently provide funding to researchers in independent and government laboratories for various research and development activities pertaining to human mutation research and the development of new technologies. Each agency, appropriately, proceeds down a slightly different path. OTA estimates that a total of about $14.3 million was spent on human mutation research in fiscal year 1985. Progress in this research could be speeded up by increased funding, though it is difficult to quantify an expected gain. It is clear also that less money spent on human mutation research will slow progress in laboratories already engaged in this research, and could deter other scientists from pursuing research in this field.

Option 1: Congress could assure that funding levels for human mutation research and closely related studies do not decline without the responsible agencies assessing the impact of funding cuts on research progress. This requirement could be expressed in appropriation, authorization, or oversight activities.

Of the several funding agencies, DOE has taken the lead in funding much of the research on new methods for mutation detection described in this report, and researchers at some of DOE's National Laboratories are among the leaders in the research. DOE also is the agency responsible for funding U.S. participation in the Radiation Effects Research Foundation, the joint United States-Japan body that continues to study the health of Japanese atomic bomb survivors.

Option 2: A lead agency for research related to detecting and characterizing mutations could be designated. The lead agency would be responsible for tracking the development of new technologies, facilitating the interchange of information among scientists developing the technologies and those in related fields, and encouraging and facilitating coordinated studies involving different subdisciplines. The lead agency also would keep Congress informed about activities in this area. DOE may be the logical choice for a lead agency.

The types of activities that a lead agency might engage in are described below. These activities are important whether or not a lead agency is designated, and Congress should consider directly encouraging them if there is no lead agency.

Tracking the Development of New Technologies

All the new technologies require improvements in efficiency before they become useful tools for studying human beings. As research proceeds, some techniques will develop more quickly than others, some will be dropped, and some may change in character, altering the kinds of mutations they can detect. The lead agency would be responsible for keeping track of these developments.

It would be useful if the "tracking" responsibility could lead to actions on the part of the lead agency that would promote the rapid and efficient development of the technologies. At some point, the technologies will be ready for feasibility testing and eventually, field testing. It would help researchers to know the stages of development of various methods, and it could help agencies make decisions about funding studies using certain methods. In addition, the lead agency could assist researchers by anticipating needs that will be common to all research programs and by encouraging efficient use of resources.

Facilitating Information Exchange Among Researchers

In 1984, DOE organized and funded a meeting that brought together for the first time many of the researchers involved in laboratory-based mutation research. This meeting is acknowledged among those who attended as a milestone for information exchange and the generation of new ideas concerning detection of heritable mutations. In fact, the ideas for some of the new methods described in this report were born at that meeting. There is a continuing need for this type of information exchange.

Keeping Congress Informed

Congress has already directed regulatory agencies to reduce exposures to environmental agents that may cause mutations. As part of its oversight of both regulatory and research activities, Congress could benefit from up-to-date information about the development of various technologies. The lead agency could report in a specified manner, e.g., a brief annual report describing the level of current research, its goals, results of completed or ongoing work, and expected near-term developments. This information could also be the basis for informing the public about mutation research. This activity will continue to be valuable to Congress through later phases of development and application of new technologies.

Feasibility and Validity Testing

After technologies pass through a phase of development to improve their efficiency and to work out technical details, a period of feasibility and validity testing will be necessary before a technology can rationally be used as a tool in a large-scale study of heritable mutations in human beings. There are some ways to make validity testing an efficient process. As an example, a "DNA library," a collection of known DNA sequences, particularly sequences carrying known mutations, could be established and maintained by one laboratory, which could make DNA sequences available to all researchers developing mutation detection technologies. This material could be used to determine whether a particular technology detects those mutations that it is designed to detect, analogous to testing chemical procedures and equipment against known chemical "standards." At a more advanced stage, new technologies might also be tested in animal experiments with a selection of mutagens known to cause specific types of mutations. If a lead agency is designated, some of these options would logically be among

that agency's responsibilities. If there is no lead agency, these functions could still be encouraged by Congress through oversight activities.

Banking Biological Samples

Biological samples, especially blood samples, are often collected during the course of medical examinations for people thought to be exposed to environmental or occupational agents. Implementation of a plan to bank those samples would facilitate human mutation studies and other related research.

Examining stored samples is not nearly so disruptive as collecting samples from a population. The very act of specifically collecting samples for a study of mutations would raise expectations that definitive information about risks would be forthcoming. Examining stored samples would avoid that human cost and could, at the same time, provide a realistic test of new technologies before they are applied to people who are anxious about the effects of environmental exposures on their genes.

Although stored samples offer many advantages, preparing human samples for storage and maintaining the stored samples is a significant task. Currently the high cost of storing samples inhibits establishment of sample banks. There has been little research directly aimed at improved methods for storing biological samples.

Option 3: Congress could encourage banking of biological material obtained from at-risk individuals and their spouses and offspring, with the objective of studying somatic and heritable mutations from these stored samples before technologies are used directly in a study of an exposed population. A centralized samples bank available to all researchers would be the most efficient means of establishing a repository of sufficient size and scope for validity testing.

Option 4: Congress could encourage the appropriate agencies (through the lead agency, if one is designated) to investigate the potential for improving technologies for storing biological samples. Where possible, funding should be encouraged for such improvements. This option is relevant to a broad spectrum of human health research, and collaboration among disciplines should be encouraged for determining the specific storage needs of different lines of research.

Field Studies

Sometime in the next 5 to 10 years, it is likely that one or more of the technologies for studying human mutations will be brought to the point of readiness for epidemiologic studies of human beings thought to be at risk for mutations. Such a study could be initiated by an independent scientist or a group of scientists involved in the development of a new technology who have identified an at-risk population; a new technology or technologies could be used in an environmental health investigation triggered by the discovery of a potentially at-risk population; or Congress could mandate a study of mutations in a specific population. Depending on the way

a study is initiated, different agencies will be drawn in, and different funding mechanisms will come into play.

Should a study be initiated by one or a group of investigators who submit a grant proposal for funding, the current system for review of research awards is probably appropriate for making scientific judgments about whether a method is ready for large-scale testing. From that point on, however, there are significant differences between a proposal to use a new technology in a human population and most other proposals. The major differences are size and money. Application of any promising technology will require that state-of-the-art methods in molecular biology be expanded from small-scale laboratory use to large-scale examination of blood samples collected from hundreds or thousands of people.

A study such as that described above would require a significant commitment of funds over a period of years, and could account for a large percentage of a granting agency's funds. The amount of money necessary can only be estimated, but it could amount to tens of millions of dollars, not a large amount in government spending, but large in comparison to most biomedical research grants, which usually range from less than $100,000 to about $500,000 annually. The necessary commitment of funds from any single agency, considering current spending for this type of research, is likely to be impossible, no matter how worthwhile the proposed study.

Option 5: Congress could consider providing specific add-on funding to finance an investigator-initiated realistic test of a promising method to study human heritable mutations.

A study mandated by Congress or an agency-initiated study using new technologies will not necessarily undergo the rigorous review and criticism that a proposal submitted to a granting agency would. Congress already has some experience in mandating epidemiologic studies. Concerns about cancer and birth defects convinced Congress to mandate studies of military veterans who were exposed to Agent Orange or who participated in atomic bomb tests. In addition, Congress has vested the Public Health Service with the authority to carry out a wide range of health investigations of exposures from toxic waste dumps through "Superfund." The role of the mandated "Agency for Toxic Substances and Disease Registry" (ATSDR) is to "effectuate and implement the health related authorities" of Superfund. ATSDR, located within the Centers for Disease Control, is a logical place for new technologies to be used as exposed populations are identified through other provisions of Superfund.

An unusual array of health problems or an apparent excess of disease among people living around toxic waste sites could trigger a study by ATSDR. Such problems could also prompt local residents to press Congress for studies to determine whether, among other things, the disorders had been caused by mutations, and whether these mutations could have resulted from chemicals in the waste site.

Some scientific societies, such as the American Society of Human Genetics, could be asked to participate in a review of the feasibility of de-

tecting possible health effects from environmental exposures. If the study is determined to be feasible, Congress and the public can be reassured that the study's findings are likely to be useful in decisionmaking. Alternatively, a decision that no study is currently feasible would underline the importance of developing and testing new methods. Reviewing the feasibility of a study may be perceived as delaying an investigation unnecessarily. However, performing a study that has little or no likelihood of answering questions about exposure and mutations has marginal value at best and would not serve the people who requested it.

Option 6: If Congress mandates a study of heritable mutations in an at-risk population using the new technologies, the mandate should include a feasibility assessment by a panel of experts before the study begins.

Option 7: Congress could require agencies to plan for a rigorous outside review by a panel of experts of any agency-initiated study using a new technology, before such a study can proceed.

Integration of Animal and Human Studies

Much of our current ability to estimate the effects of various external agents on human beings is derived from animal studies. In the future, animals will continue to be used to test for mutagenicity and, ideally, to predict effects in human beings. Right now, the available methods and body of data provide an inadequate basis for making predictions from animal results to human effects. The new technologies described in this report for detecting heritable and somatic mutations can be applied in both animal systems and in human beings. This information and information from currently available animal systems could be used in an integrated fashion to study relationships between somatic, germinal, and heritable mutations, and to pursue the development of extrapolation models for predicting effects in human beings.

The kind of integrated research necessary will not occur spontaneously if the current pattern holds. There are few researchers engaged in studies that integrate comparable information from different systems for the purpose of elucidating relationships among such systems. Improvements in extrapolation from animal to human data and from somatic to heritable mutations await efforts to encourage and coordinate the appropriate research. Single laboratories or centers are unlikely to be able to perform all the different tests necessary to develop and test extrapolation models, making coordination among laboratories essential.

The National Toxicology Program (NTP) is the center of Federal mutation research using experimental animals, funding research grants and contracts nationally and internationally. The National Center for Toxicological Research, a laboratory of the Food and Drug Administration that is part of NTP, has facilities and experience to carry out large-scale animal tests. EPA also has a genetic toxicology program, and it is actively pursuing development and application of methods for studying human somatic mutations. DOE's National Laboratories have directed or carried out the

majority of large-scale animal studies of mutation rates and mechanisms, and this experience, as well as the advanced technology that these laboratories have developed for sorting and studying chromosomes and cells, will be useful in improving methods for extrapolation. These three agencies could encourage the development of methods for linking information from animal studies to somatic and heritable mutation risks in humans.

Option 8: Congress could encourage studies of somatic, germinal, and heritable mutations in experimental animals using both currently available and new technologies. Further, research funding agencies should encourage animal studies directed at identifying the mechanisms of mutagenesis and elucidating relationships between mutagenic potencies in animals and in human beings.

NOTE: Copies of the full report "Technologies for Detecting Heritable Mutations in Human Beings" can be purchased from the Superintendent of Documents, U.S. Government Printing Office, Washington, DC 20402, GPO stock No. 052-003-01037-2.

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